Translation

PCT/EP2003/009453

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference M/43128-PCT	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP2003/009453	International filing date (*day/month/year*) 26 August 2003 (26.08.2003)	Priority date (*day/month/year*) 26 August 2002 (26.08.2002)
International Patent Classification (IPC) or national classification and IPC C12P 13/04		
Applicant BASF AKTIENGESELLSCHAFT		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 10 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of _____ sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☒ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
24 March 2004 (24.03.2004)	18 November 2004 (18.11.2004)
Name and mailing address of the IPEA/EP Facsimile No.	Authorized officer Telephone No.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/EP2003/009453

I. Basis of the report

1. With regard to the **elements** of the international application:*

☐ the international application as originally filed

☒ the description:

pages 1-40, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

☒ the claims:

pages 1-16, as originally filed
pages _____, as amended (together with any statement under Article 19
pages _____, filed with the demand
pages _____, filed with the letter of _____

☒ the drawings:

pages 1/3-3/3, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

☒ the sequence listing part of the description:

pages 1-130, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____
☐ the claims, Nos. _____
☐ the drawings, sheets/fig _____

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rule 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

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IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:

See Supplemental sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☒ all parts.

☐ the parts relating to claims Nos. _____

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/EP 03/09453

I. Basis of the report

1. This report has been drawn on the basis of *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.)*:

1). **Priority**

The present application is fully supported by the priority document DE 10239082 and hence the priority date 26 August 2002 is valid.

Consequently, documents WO 02/097096 (publication date 5 December 2002) and "J. Biotechnol.", (publication date 12 June 2003), both marked as P documents in the international search report, are not taken into consideration in the international examination procedure.

However, as a precaution the applicant's attention is drawn to the fact that document WO 02/097096 was filed before the filing date of the present application and could therefore play a role in the assessment with regard to novelty during the regional procedure before the EPO.

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: IV.3

2). Lack of unity of invention

The Examining Authority has found that the international application comprises a number of inventions or groups of inventions not linked by a single general inventive concept (PCT Rule 13.1), that is to say:

I: Claims 1-14 and 16;
II: Claim 15.

The reasons are as follows:

The aim of **invention 1** is to provide a method for the fermentative production of a sulphur-containing fine chemical (L-methionine), which was achieved by the use of a culture of coryneform bacteria in which at least one heterologous nucleotide sequence coding for a protein with homoserine-O-acetyl-sulfhydrolase (metY) activity is expressed.

The aim of **invention 2** is to provide an L-methionine-containing animal feed additive from fermentation broths. This was accomplished by cultivating and fermenting *any* microorganism producing L-methionine, removing water and biomass and drying the resulting fermentation broth. Invention 2 contains no reference to invention 1.

Since inventions 1 and 2 solve different problems the solutions thereto are likewise different and not linked by a single general inventive concept.
The special technical features each of the inventions

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: IV.3

contributes to the prior art (use of a specific microorganism in invention 1; preparation of a fermentation broth of any microorganism in invention 2) likewise differ and therefore fail to meet the unity of invention requirement.

Since the PCT procedure was brief, and owing to the fact that the additional search and substantive examination did not entail much outlay and that claim 15 in any event does not appear to be novel (see below), the applicant is not invited to pay additional fees in the international procedure.

However, this point will be raised in the regional procedure before the EPO.

Moreover, as regards the unity of the invention, reference is made to the last paragraph of item 3.e) below.

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V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	4	YES
	Claims	1-3, 5-16	NO
Inventive step (IS)	Claims		YES
	Claims	1-16	NO
Industrial applicability (IA)	Claims	1-16	YES
	Claims		NO

2. Citations and explanations

3.a) The international search report citations are numbered as follows:

D1: WO 02/18613 A (DEGUSSA) 7 March 2002 (2002-03-07)

D2: WO 02/10206 A (DEGUSSA) 7 February 2002 (2002-02-07)

D3: WO 02/097096 A (DEGUSSA) 5 December 2002 (2002-12-05)

D4: WO 02/10209 A (DEGUSSA) 7 February 2002 (2002-2-07)

D5: HWANG BYUNG-JOON ET AL: "Corynebacterium glutamicum utilizes both transsulfuration and direct sulfhydrylation pathways for methionine biosynthesis" JOURNAL OF BACTERIOLOGY, Vol. 184, No. 5, March 2002 (2002-03), pages 1277-1286, XP002269798 ISSN: 0021-9193

D6: REY DANIEL ALEXANDER ET AL: "The putative transcriptional repressor McbR, member of the TetR-family, is involved in the regulation of the metabolic network directing the synthesis of sulfur containing amino acids in Corynebacterium glutamicum." JOURNAL OF BIOTECHNOLOGY, Vol. 103, No. 1, 12 June 2003 (2003-06-12), pages 51-65,

XP001152914 ISSN: 0168-1656 (ISSN print)
D7: WO 93/17112 A (GENENCOR INT) 2 September 1993 (1993-09-02).

As already stated in item 1) above, documents D3 and D6 are not taken into consideration for the purpose of the PCT procedure.

3.b) The present application:

Claims 1-14 of the present application concern a method for the fermentative production of a sulfur-containing fine chemical (L-methionine) wherein a culture of coryneform bacteria is used in which a heterologous nucleotide sequence coding for a protein with an O-acetyl-homoserine-sulfhydrolase (metY) activity is expressed.

Claim 15 concerns a method for producing an L-methionine-containing animal feed additive from fermentation broths by cultivating and fermenting *any* microorganism producing L-methionine, removing water and biomass and drying the resulting fermentation broth.

Claim 16 makes use of the microorganisms used in the method according to claims 1-14, and hence claim 16 is considered a multiple-step method incorporating claims 1-14.

The present application contains the following defects (**PCT Article 5 and 6**) which are significant for the substantive examination:

-) the expression "sulphur-containing fine chemical" in the claims (especially claim 1) and the description is so broad and undefined that it leaves the subject matter for which protection is sought in the claim unclear. In addition, it is clear from the description and the examples that only L-methionine is produced;

-) the feature "less than 100 % sequence homology" in claim 3 is meaningless because the scope of the claim includes *all* the sequences apart from the metY-coding sequence from *Corynebacerium glutamicum* ATCC 13032. Consequently, claim 3 is too broad, vague and undefined and for the purpose of the substantive examination is hence interpreted in its broadest form.

-) Claims 5 and 6 contain so-called functional definitions, that is to say, a feature is defined in terms of its function, that is, the result to be obtained by it. In the present case this objection pertains to the "homologous sequences", which are defined only in terms of their function and therefore require the user of the patent to make an unreasonable effort when testing for these functions.

The same objection is also raised with regard to claims 10-12, wherein the above kind of functional feature, that is, "... and is mutated in such way as to ..." leaves the subject matter for which protection is sought completely vague and undefined.

Claims 5, 6 and 10-12 are therefore vague and undefined and, in the light of the description, much

too broad.

Since the term "homologous" is not defined per se, claims 5 and 6 are likewise interpreted as broadly as possible for the purpose of the examination.

3.c) **Brief discussion of the prior art documents**:

Document **D1** discloses the production of "sulfur-containing fine chemicals" (especially L-lysine and L-methionine) using coryneform bacteria in which the metY gene was overexpressed (see in particular page 10, claim 10 and example 6).

Since the gene used in document D1 (preferably from *C. glutamicum* ATCC 13032) can also have sequence modifications - see, for example, page 5 and claims 1 and 5 of document D1 - document D1 is likewise relevant to the present claims relating to a (non-specific) sequence homology. Further possible genetic modifications are disclosed on pages 13 and 14 of document D1.

Document D1 further discloses methods for the production of an L-methionine-containing feed additive from fermentation broths, which comprises the same steps as the present claim 15 (see claim 25 in document D1). Document D1 is therefore prejudicial to the novelty of claims 1-3 and 5-16 of the application and relevant to the assessment of claims 1-16 with regard to inventive step.

In this regard the examiner wishes to point out that the content of document D1 was not accurately reproduced on page 2 of the present description.

Documents D2 and D4 both describe the production of methionine using coryneform microorganisms wherein, the, *inter alia*, metY gene is (over)expressed (see, for example, page 37 and claim 15 in document D1, and claim 16 in document D4).

In addition, methods for producing an L-methionine-containing feed additive from fermentation broths are disclosed, which comprise the same steps as the present claim 15 (see claim 19 in document D2 and claim 20 in document D4). Documents D2 and D4 are therefore likewise prejudicial to the novelty of the present claims 1-3 and 5-16.

Document **D5** describes the role of the metY gene in the biosynthesis of methionine in transgenic bacteria and is therefore important for the assessment with regard to inventive step.

Document **D7** concerns the biosynthetic production of amino acids in microorganisms. Example 4 describes the production of methionine using bacteria (for example, *E. coli* and *C. glutamicum* transformed by a gene coding for homocysteine-methylase). Page 4 states that homocysteine methylase is another name for O-acetyl-homoserine-thiol-lyase, that is, the enzyme used in the present application. Although the metY gene is not mentioned, document D7 is relevant to the assessment with regard to inventive step.

3.d) Novelty (PCT Article 33(1) and (2)):

As already stated in item c) above, the subject matter of claims 1-3 and 5-16 is not novel in the

light of documents D1, D2 and D4.

3.e) Inventive step (PCT Article 33(1) and (3)):

Although claim 4 is novel insofar as it does not encompass any non-specific sequence homologies, it is not considered to involve an inventive step.

The present application differs from the prior art by virtue of the selection of the microorganisms listed in claim 4. It is not clear what problem is solved by these microorganisms as compared with the methods known from the prior art.

The applicant's attention is again drawn to the fact that the use of transgenic bacteria containing a heterologous nucleotide sequence coding for a protein with an O-acetyl-homoserine-sulfhydrolase (metY) activity in the production of methionine is known (see documents D1, D2, D4, D5 and D7). Although the bacterial strains listed in claim 4 are not mentioned in the above documents, these strains appear to be known for the fact (see description, for example pages 13-15, of the present application) that they have an O-acetyl-homoserine-sulfhydrolase (metY) activity. It is therefore not clear what advantage there might lie in using the above microorganisms as opposed to those known from the prior art.

The results of a comparative test disclosed on page 40 of the present description show only that the microorganism transformed with the metY gene has a higher activity. However, this is already known from the prior art (see, for example, page 34 of document

D1).

No inventive step can therefore be recognized for the subject matter of claim 4.

As a precaution the examiner also wishes to point out that, should the inventive step be considered to consist in the selection of these specific microorganisms, the selection of each individual microorganism would constitute a separate invention, such that claim 4 would result in 27 different inventions and hence not meet the unity of invention requirement.

6). Industrial applicability (PCT Article 33(1) and (4)):

The subject matter of claims 1-16 is industrially applicable.